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STUDIES ON *EUTOBRILUS HEPTAPAPILLATUS* (NEMATODA: TOBRILIDAE) THE PREDOMINANT NEMATODE INHABITING THE BOTTOMS OF LAKES ALBERT AND ALEXANDRINA, SOUTH AUSTRALIA

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Summary

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Eutobrilus heptapapillatus, a cosmopolitan fresh water nematode has been isolated from the bottoms of Lakes Albert and Alexandrina where it comprises up to 87% and 85% of the nematode population respectively. The environment at the bottoms of the lakes in which these nematodes live is described and measurements of males and females from each of these environments are compared with those of a South African population. There are significant differences in tail length between the Australian and South African populations. Egg laying in the Australian population has been observed and is described. The presence of crystalloid structures in these nematodes has been noted and the possibility of their occurrence being associated with increased salinity is discussed.

KEY WORDS: *Eutobrilus heptapapillatus*, nematodes, Lake Albert, Lake Alexandrina, sediment, eggs, morphology, measurements, crystalloids.

Introduction

The nematode *Eutobrilus heptapapillatus* (Joubert & Heyns, 1979) Tsalolikhin, 1981 has a world-wide distribution in a range of freshwater habitats. In South Australia this nematode occurs at various sites on the shores of Lake Alexandrina and Hindmarsh Island at the mouth of the Murray River (Nicholas *et al.* 1992) and was the most common species extracted from a sample dredged from a depth of 3 m at the southern end of Lake Alexandrina. To date no studies have been published on measurements of this nematode nor of its presence or absence at the bottom of the adjacent Lake Albert.

In this paper I compare measurements of males and females of *E. heptapapillatus* from Lakes Albert and Alexandrina with those from South African populations (Swart & Heyns 1988). I also describe their habitats and their proportions to other nematode species found in these habitats, as well as the percentage of *E. heptapapillatus* containing crystalloid inclusions.

Materials and Methods

Collection of material

Samples were collected using a benthic grab from the bottoms of Lakes Albert and Alexandrina at the following localities. For Lake Alexandrina the collecting site was at the navigation marker No. 84 (Fig. 1 site [1]). The Lake Albert collecting site was 2-3 km off shore from the town of Meningie with compass bearings 135° on the town's water tower, 285° on trees on the Coorong side of the lake, 205° on a headland on the port side and 85° on a barren hill top on the starboard side (Fig. 1 site [2]). In each case the contents of the benthic grab were placed in a plastic bag and stored in a cooled insulated container.

The dry weight of the sediment was determined by allowing it to gravitate from the water included in the benthic sample in a graduated cylinder. The supernatant was removed by suction and the sediment was then spooned into a weighed beaker which was placed in an incubator at 40°C. Dehydration was maintained until a constant weight was reached.

Membrane (0.2 μm) filtered water samples from the lakes were taken simultaneously with the sediment samples taken with the benthic grab. Salinity was calculated from electrical conductivity (Nicholas *et al.* 1992) and a range of elements was analysed using the technique of Zarcinas and Cartwright (1983). Particle size of these samples was measured using various techniques as described by Beech (Nicholas *et al.* 1992).

A large plastic container was filled with lake water from the sampling site and this water was used to dilute the samples during the sieving procedures used to separate the nematodes. This consisted of passing the samples through 2 mm, 850 μm, 710 μm, 250 μm, 120 μm and 90 μm sieves. In samples containing much sand, further sieving through 75 μm, 53 μm and 38 μm sieves was undertaken. However, in the case of Lake Albert samples, the sediment which passed through the 90 μm sieve would have blocked the remaining three sieves. Accordingly, the material obtained on the 120 μm and 90 μm sieves was diluted to facilitate microscopic observation and aliquots were examined under the dissecting microscope. The nematodes were picked out alive on mounted eyelashes, their movement indicating their presence in the sample.

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They were placed in a test tube in a small volume of filtered lake water and an equal volume of boiling double strength FA 4: 1 solution (20 ml 40% formaldehyde and 2 ml glacial acetic acid in 78 ml of distilled water) (Hooper 1986) was added to the shaken suspension of nematodes. These specimens were processed to pure glycerol using Seinhorst's (1959) method and mounted in anhydrous glycerol on slides sealed to a coverslip by molten paraffin as described by De Maeseneer and D'Herde (1963). Nematodes fixed and processed into glycerol in this manner were photographed with Ilford Pan F film. Living nematodes, for example females laying eggs, were photographed using Ilford Delta 400 film. These nematodes were observed and photographed using a Vanox AHBT research microscope equipped with bright field and interference contrast (Nomarski) optics.

Results

The water environment

Lake Albert is a relatively large body of water about 16 km x 10 km connected to Lake Alexandrina, which is approximately 30 km x 15 km, by a narrow channel of water (Fig. 1).

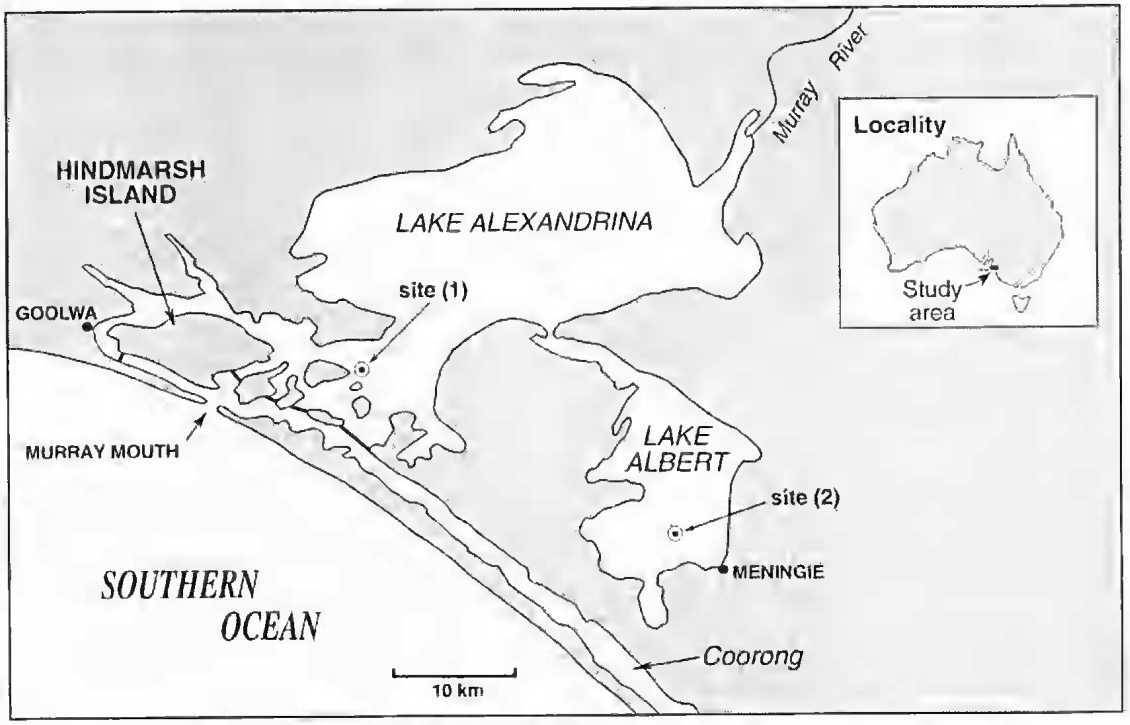

Fig. 1 Map showing the location of collecting sites (1) and (2) in Lakes Alexandrina and Albert respectively.

TABLE 1. *Analyses of major soluble ions in water from the shores of Lakes Alexandrina and Albert sampled on the same day at a six monthly interval and from water sampled from the middle of Lake Albert at a later date.*

Date (Site)	Locality	Na	Cl	Ca	Mg	K	S	E.C.*[1] ds m^{-1}	TSS*[2] %
		mgl^{-1}							
29 April 1993	Alexandrina	48	82	15	11	5	6	0.42	0.03
(shore)	Albert	214	348	38	33	12	22	1.6	0.10
22 Oct. 1993	Alexandrina	54	105	9	8	3	7	0.4	0.03
(shore)	Albert	200	375	34	31	10	22	1.5	0.10
20 May 1994 (mid-lake)	Albert	188	265	34	28	10	16	1.23	0.06

[1] E.C. = electrical conductivity (deci-siemens m^{-1})
[2] TSS = total soluble salts (estimated percentage)

The results of the analyses of water collected from Lakes Albert and Alexandrina are given in Table 1. From these results it can be seen that there is mostly a three-to four-fold difference in the total soluble salts in water samples collected from the shores of the two lakes on the same day. These differences in the major ions persisted in water samples taken six months later (Table 1).

The sediment environment

The surface 15 cm of the soil at the bottom of Lake Albert consists of a slimy sediment, largely composed of clay which made up 48-61% of samples of this surface sediment or slime taken from various parts of the lake as the top component of core samples (Taylor & Poole 1931).

It was estimated that only 1/6th of the sediment from Lake Albert consisted of solid material. This material comprised 67% clay, 25% silt, 5% fine sand and less than 1% coarse sand.

The nematode

The most common nematode in the Lake Albert sediment, *Eutobrilus heptapapillatus*, comprised up to 87% of the nematode population; the remainder mostly consisted of monhysterids. Similarly the benthic sample from Lake Alexandrina comprised up to 85% *E. heptapapillatus*.

The ratios of larvae, males and females were similar in two different collections from Lake Albert. In one instance an aliquot containing 137 nematodes had 43% larvae, 16% males and 41% females. In the other harvest the ratios were 39% larvae, 23% males and 38% females. In an aliquot containing *E. heptapapillatus* from the Lake Alexandrina benthic sample, the ratios were 51% larvae, 37% males and 12% females. An obvious difference between these two populations was the presence of crystalloid inclusions (Fig. 2) in 32% of the nematodes from Lake Albert whereas none was observed from the Lake Alexandrina sample.

Comparison of populations of E. heptapapillatus.

Specimens from both lakes were measured and compared with each other and with those from South Africa (Swart & Heyns 1988). It can be seen (Table 2) that the males of these three populations are similar in many respects. For example, they are of similar length, have the same body width at the anus and have similarly-sized copulatory spicules. Differences in maximum body width and pharynx length could not be analysed statistically due to the absence of certain measurements of the South African population. There is, however, a significant difference in tail length ($P<0.001$) between the Australian populations (Lake Albert with a mean of 179 μm and Lake Alexandrina with a mean of 173 μm) and the South African population (mean of 244 μm). This significant difference in tail length is not so pronounced ($P<0.01$) in the females of these populations (Table 3). The males of *E. heptapapillatus* (Figs 3, 4) have a diorchic reproductive system consisting of a pair of testes, a vas deferens and ejaculatory duct connecting with the copulatory spicules. The most obvious components of the male's accessory structures are the seven supplementary organs (Fig. 4) from which its specific name is derived.

The distances between these supplementary organs in the South African population have been measured (Swart & Heyns 1988) and so can be compared with the Australian populations. Measurements of the Australian populations are expressed as percentages of the sum of these distances rather than as direct measurements. This is because direct measurements using coiled and uncoiled nematodes revealed a significant difference in the mean value of the distance between supplements in the coiled (27.9 μm) and the uncoiled (41.4 μm) ($P<0.001$). However, when these distances were expressed as percentages of the sum of the distances between supplements, there was no

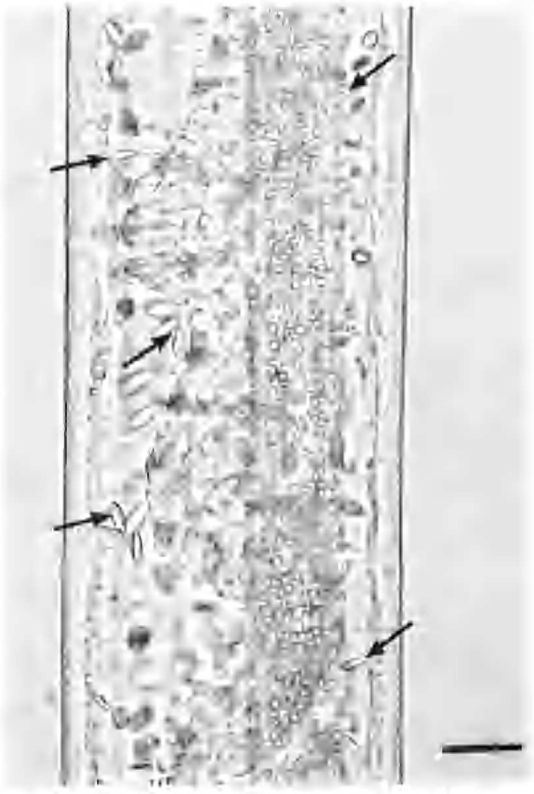

Fig. 2. Photograph of part of an adult female *Eutobrilus heptapapillatus* that had been maintained in shallow distilled water in a Petri dish for over a month prior to being photographed. Bright field optics showing the presence of numerous crystalloid bodies (small arrows). Scale bar = 20 μm.

TABLE 2. *Comparison of measurements of different populations of males of* Eutobrilus heptapapillatus.

All measurements μm	Tsitsikama Forest Cape Province, South Africa (Swart & Heyns 1988) n = 7		Lake Albert South Australia (present study) n = 5			Lake Alexandrina South Australia (present study) n = 5		
Parts measured	Range	Mean	Range	Mean	SD	Range	Mean	SD
Body length (L)	1550-2120	1920	1873-2000	1931	±57	1800-1990	1896	±68
Max. body width		53*	64-77	71	±5	60-70	66	±4.1
Pharynx length		369*[1]	305-327	311	±9	270-315	290	±18
Tail length	211-300	244	168-193	179	±9	140-192	173	±20
Body width at anus		38*[1]	36-41	38	±2.7	32-40	38	±3.6
Spicule	48-57	53.1	50-55	54	±2.2	52-56	53	±1.8
Gubernaculum	35-39	37.0	23-36	31	±5.7	30-36	33	±2.7
De Man's indices a	32.1-41.3	36.2	25.6-30	27	±1.8	28.1-31.3	29	±1.3
De Man's indices b	5.1-5.3	5.2	5.7-6.6	6.2	±0.3	6.1-7.0	6.6	±0.4
De Man's indices c	6.2-8.8	7.9	10.4-11.6	10.8	±0.5	9.9-12.9	11.1	±1.1
De Man's indices c'	5.8-8.0	6.5	4.4-5.3	4.7	±0.4	4.4-4.8	4.6	±0.2

[1], [2], * calculated from data of Swart & Heyns (1988)

TABLE 3. *Comparison of measurements of different populations of females of* Eutobrilus heptapapillatus.

All measurements μm	Tsitsikama Forest Cape Province, South Africa (Swart & Heyns 1988) n = 21		Lake Albert South Australia (present study) n = 5			Lake Alexandrina South Australia (present study) n = 5		
Parts measured	Range	Mean	Range	Mean	SD	Range	Mean	SD
Body length (L)	1720-2280	2040	2182-2254	2209	±30	1940-2200	2094	±128
Max. body width		60*[1]	82-95	88	±6	65-82	74	±8.2
Pharynx length		385*[1]	336-382	358	±18	285-345	320	±22
Tail length	228-337	292	218-268	242	±20	224-296	261	±27
Body width at anus		34*[1]	41-45	42	±2	36-40	38	±2
De Man's indices V	39-48	42	43-47	45	±1	33-48	40	±6.0
De Man's indices a	26-41	34	23-27	25	±2	26-34	28	±3.2
De Man's indices b	4.8-5.9	5.3	5.9-6.6	6.2	±0.3	6.1-6.9	6.6	±0.3
De Man's indices c	6.1-9.0	7.1	8.2-10.2	9.2	±0.8	7.4-8.7	8.1	±0.5
De Man's indices c'	6.8-10.0	8.7	5.3-6.5	5.8	±0.5	6.2-7.4	6.8	±0.4

[1], [2], * calculated from data of Swart & Heyns (1988)

significant difference ($P>0.05$) between the means of coiled (14.3%) and uncoiled (also 14.3%) nematodes. Thus expressing distances between supplements as percentages rather than actual measurements when making comparisons between nematodes that are coiled into various shapes provides a standardised measure for differently-coiled nematodes.

Measurements of the Australian populations were combined to obtain a pooled estimate of the means and standard deviations. Because these values appeared normally distributed, standard deviations of the South African values were calculated assuming a normal distribution (Table 4).

The positions of the Australian population male supplementary organs differ in some respects from those of the South African population (Table 4) although these differences are not significant except for the S_2 and S_3 ($P<0.01$). These differences appear minor compared with the similarities that exist between the populations. Thus the females (Table 3) are of similar length and have a similar vulval position although the South African population appears narrower with a longer pharynx and a significantly longer tail. The females of this species (Figs 5, 6) are didelphic and amphidelphic. The genital tract (Fig. 6) consists of ovary, short oviduct, pars dilatata and uterus that may contain oval-shaped sperm.

Egg laying

The laying process was observed in a specimen collected the previous day from Lake Alexandrina. It was in a sitting drop slide in filtered Lake Alexandrina water (0.2 μm membrane). Egg laying took place at 23°C and was very rapid, the actual emergence of the egg being completed in several seconds. The whole process was filmed (Fig. 7) (Ilford XP 1 400 film). The egg is shown moving from the pars dilatata into the uterus (Fig. 7A, B) and from there into the vagina (Fig. 7C, D). During the final stages of laying, the egg moves from the vagina to the exterior through the vulva (Fig. 7E, F, G, H). The egg which is oval (ellipsoidal) within the nematode assumes a spherical shape soon after laying (Fig. 8). It has a mean diameter of 70.8 μm (±2.9 SD) including the shell which has a mean thickness of 8.4 μm (±0.6 SD). This compares with *in utero* measurements of fixed material of 73.5 μm x 48.9 μm including an egg shell thickness of 5.5 μm (Swart & Heyns 1988).

Discussion

Over 60 years ago Taylor and Poole of CS&IR Division of Soil Research (now CSIRO Division of Soils) published the results of a soil survey of the bed of Lake Albert (Taylor & Poole 1931). This work resulted from a request by the appropriate branches of both the State

TABLE 4. *Comparison of measurements of distances between supplementary organs, expressed as percentages of the sum of the distances between supplements, in different populations of males of* Eutobrilus heptapapillatus.

Parts measured			Tsitsikama Forest Cape Province, South Africa (Swart & Heyns 1988) n = 5			Lake Albert South Australia (present study) n = 5			Lake Alexandrina South Australia (present study) n = 6		
			Range	Mean	SD*	Range	Mean	SD	Range	Mean	SD
Cloaca	‣	S7	7.6-11.4	9.3	±1.6	4.7-11.8	8.5	±2.6	9.1-11.8	9.8	±1.1
S7	‣	S6	7.6 9.2	8.3	±0.7	6.0-9.9	8.1	±1.4	8.7-12.3	10.3	±1.3
S6	‣	S5	8.8-11.3	10.2	±1.1	9.7-12.2	11.1	±1.2	10.3-13.0	11.6	±0.9
S5	‣	S4	16.2-17.8	17.4	±0.7	16.5-23.1	20.1	±2.8	17.0-21.7	19.1	±1.6
S4	‣	S3	14.4-17.6	15.6	±1.4	13.2-15.3	14.3	±1.0	12.3-15.3	13.6	±1.1
S3	→	S2	19.9-21.6	20.8	±0.7	15.3-19.7	17.2	±1.7	15.4-18.2	16.7	±1.0
S2	‣	S1	16.4-20.3	18.4	±1.6	18.6-25.0	20.7	±2.5	15.1-20.8	18.9	±2.0

* = estimated using sample size and range and assuming normal distribution

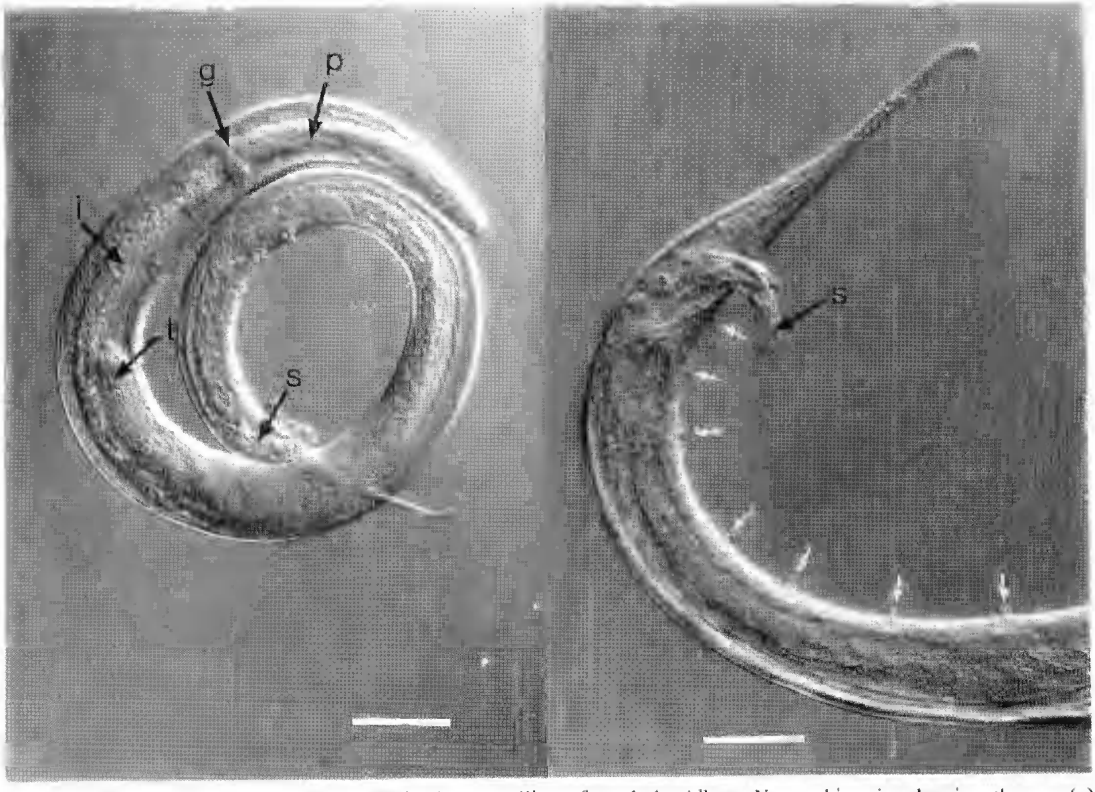

Fig. 3. Photograph of an adult male *Eutobrilus heptapapillatus* from Lake Albert. Nomarski optics showing pharynx (p), intestine (i), pharyngeal glands (g), testis (t), retracted copulatory spicules (s). Scale bar = 100 μm.

Fig. 4. Photograph of an enlarged portion of an adult male *Eutobrilus heptapapillatus* from Lake Albert. Nomarski optics showing the seven supplementary organs (small arrows) and the everted copulatory spicules (s). Note relatively short tail. Scale bar = 50 μm.

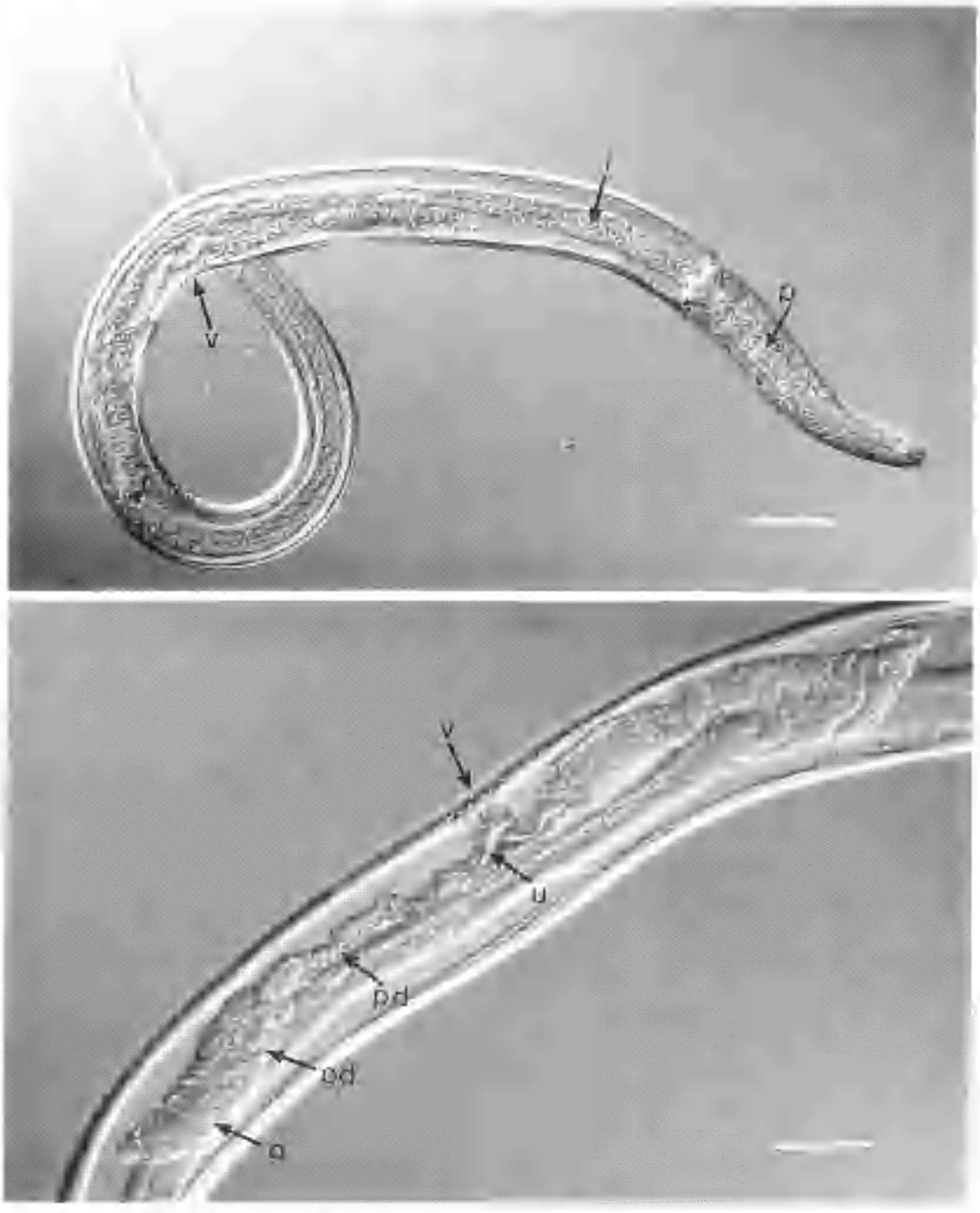

Fig. 5. Photograph of an adult female *Eutobrilus heptapapillatus* from Lake Albert. Nomarski optics showing pharynx (p), intestine (i) and vulva (v). Scale bar = 100 μm.
Fig. 6. Photograph of an enlarged portion of an adult female *Eutobrilus heptapapillatus* from Lake Albert. Nomarski optics showing the didelphic reproductive system consisting on each side of ovary (o), short oviduct (od), pars dilatata (pd), uterus (u) and vulva (v). Scale bar = 50 μm.

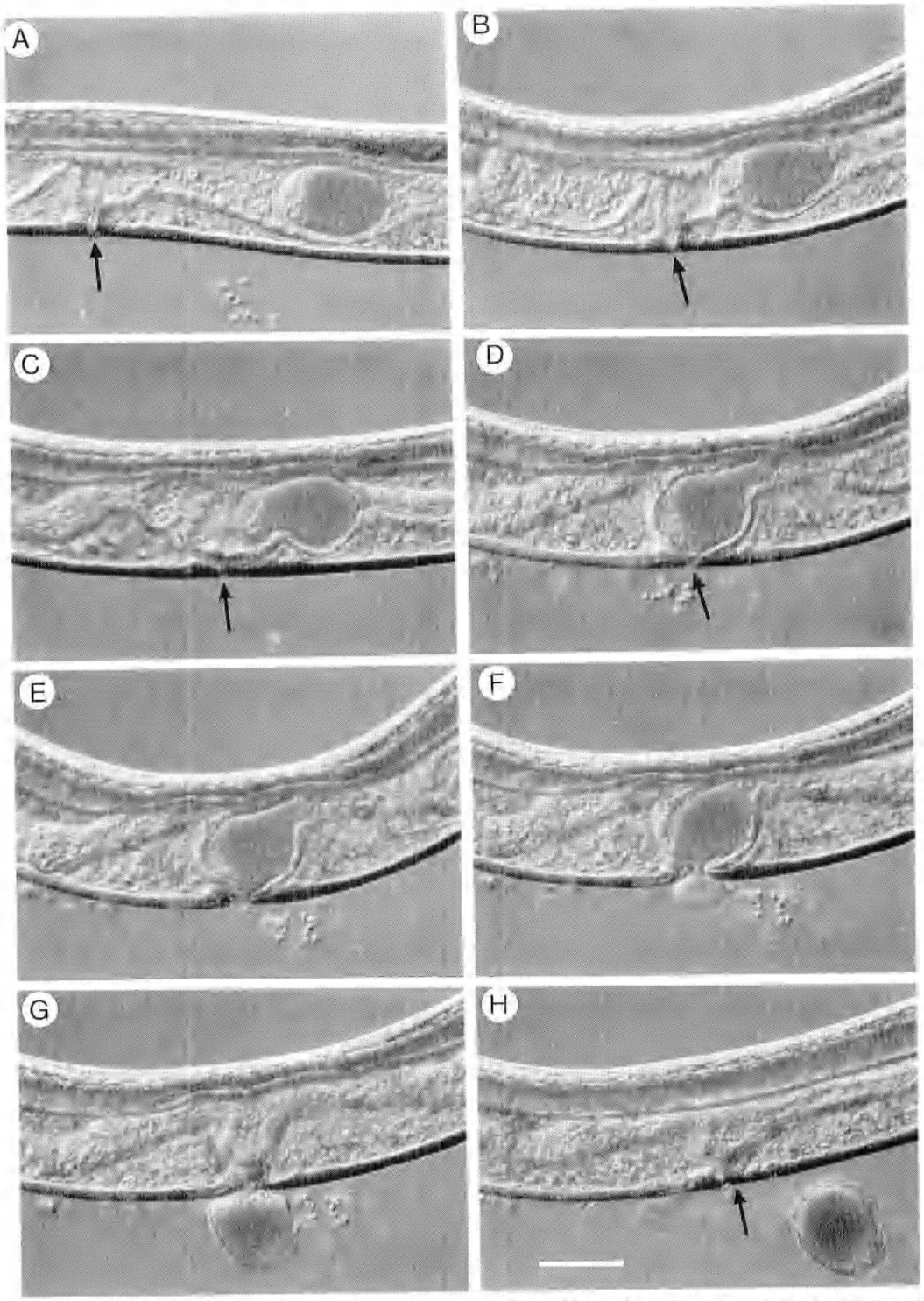

Fig. 7. Photographic sequence of egg laying in *Eutobrilus heptapapillatus*. Nomarski optics. Arrows indicate vulval opening. A., B. Movement of the egg from the pars dilatata to the uterus. C., D. Movement of the egg from uterus to vagina. E., F., G. The process of laying as the egg passes from the vagina to the exterior via the vulva. H. The newly laid egg. Scale bar = 50 μm.

and Commonwealth Governments regarding the feasibility of using the soil at the bottom of the lake for agriculture after it had been drained. Taylor and Poole (1931) showed that the drained lake would be unsuitable for agricultural purposes. Thus, at a time when the clearing of land was in full swing, these workers were able to show, as a result of their soil survey, conducted under difficult conditions, that drainage of this lake would have been a costly mistake. Furthermore, their detailed results (Taylor & Poole 1931) provided valuable information on which to base further studies of the lake's benthos.

Taylor & Poole (1931) reported that Lake Albert was once connected to the saline waters of the Coorong by an ancient river channel which persists today as a lagoon extending from the southern side of the lake (Fig. 1). Thus, although Lake Albert is not now flushed through by waters of the River Murray, as is Lake Alexandrina, it may once have been when the river was in flood.

The barrages at the mouth of the River Murray were not built until 1940 so that at the time of Taylor's and Poole's survey during March to April 1930 when the river was running low, Lake Alexandrina had become very saline as a result of incoming sea water. Thus water from Lake Alexandrina was making the water from Lake Albert more saline and "was not potable for humans and taken unwillingly by stock until accustomed to it" (Taylor & Poole 1931). Today, due to the presence of the barrages, Lake Alexandrina is much less saline than it was and although the quantities of soluble salts contained in its water can and do vary from time to time depending on river flushings, it is clear from the samples collected on the same day from both lakes at a six-monthly interval (Table 1) that Lake Albert has a higher concentration of soluble salts than Lake Alexandrina during normal river flow. Nicholas *et al.* (1992) have shown that the concentration of soluble salts in water collected from sites on the shore of Lake Alexandrina varies from month to month and hence it was necessary to collect water from the two lakes on the same day so that valid comparisons could be made.

It is interesting to speculate whether or not the presence of crystalloid bodies observed in *E. heptapapillatus* from Lake Albert but not in specimens of the nematode collected on the same day from Lake Alexandrina when its soluble salt values were low, might be associated with increased salinity in these lakes. Crystalloid bodies were found in *E. heptapapillatus* collected from Lake Alexandrina both at the water's edge and from the bottom of the lake in 97% of the nematodes examined (Bird *et al.* 1991) but no correlation was made with the salinity of the lake at that time. However, examination of these data shows that the concentrations of sodium and chloride ions (Table 2 - Nicholas *et al.* 1992) were greater than those obtained from this lake during the present study (Table 1). Furthermore, contrary to our findings that nematodes maintained in aquaria over a period of two months "appeared to be free of crystalloids," I have found that there was an almost three-fold increase in both numbers of *E. heptapapillatus* and their crystalloid content when mud from Lake Albert was placed in an aquarium tank, covered with Lake Albert water and left for four months with occasional aeration. Under these conditions, the ratio of larvae to females to males was 85 : 12 : 3 of which 93% contained crystalloids. The large number of larvae present would probably be due to relatively recent hatching from eggs and the low number of adults to lack of food. The increase in the percentage of crystalloids present from 32% to 93% in nematodes kept in an aquarium tank for four months would probably be due to an increase in the concentration of soluble salts due to evaporation from the tank. This particular batch of Lake Albert water (collected on 20 May 1994) had initial sodium and chloride ion readings of 188 and 265 mg l^{-1} respectively (Table 1) which are apparently high enough to induce the development of crystalloids.

The occurrence and possible functions of crystalloids in a number of genera of aquatic free-living nematodes have been recorded by various workers (Bird *et al.*

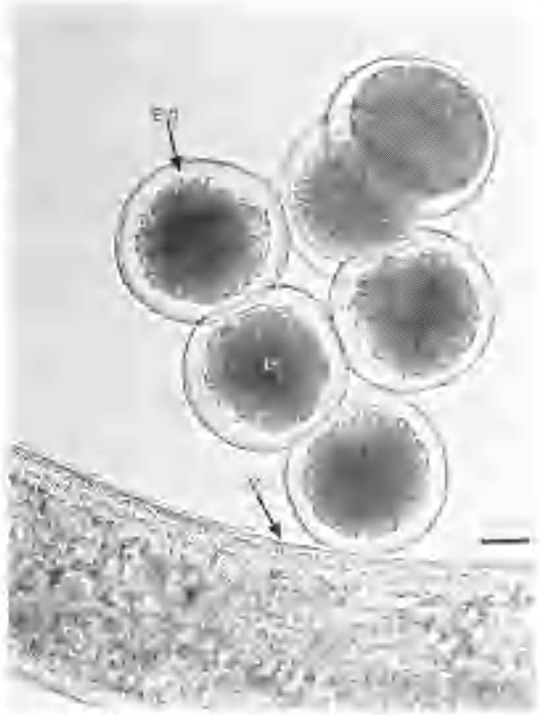

Fig. 8. Photograph of freshly laid eggs of *Eutobrilus heptapapillatus*. Bright field optics showing vulva (v) and spherical eggs (e) with their relatively thick shells (es). Scale bar = 20 μm.

1991) but as yet no clear-cut evidence for their function has been obtained.

Clearly further research is required to test whether these crystalloid structures are produced in the nematodes in response to changes in their environment and whether or not they are a manifestation of a diseased state, since they appear to be associated with small regular particles that resemble icosahedral viruses in some respects (Bird *et al.* 1991).

I think that this nematode which predominates in the sediment at the bottom of these lakes is *E. heptapapillatus* although, as will be discussed below, there are some differences between the Australian and South African populations. It has all the characteristics of its Subfamily (Eutobrilinae) described by Tsalolikhin (1983) namely, hedgehog-like supplementary organs (Fig. 3), a muscular vagina and well-differentiated female genital system (Fig. 5) and well-developed and rounded pharyngeal glands. Similarly, it is less than 3.5 mm in length and it has the described species characteristics of a cuticle without pronounced annulations, head bristles that do not exceed 14 μm and, most obvious of all, males with seven supplementary organs. It does not quite fit Tsalolikhin's key in having females whose tails are not ten times greater than the body width at the anus (Table 3) but this also applies to the South African specimens (Swart & Heyns 1988). However, these various morphological differences are likely to be reflections of variability between different populations of this species of nematode rather than suggesting that the Australian and South African nematodes are different species.

Another apparent difference is that the egg, although oval-shaped when within the female, becomes round when laid (Figs 7, 8). However, it seems likely that egg measurements in the past may have all been made while the eggs were within the female in fixed material. The transition from oval within the female to spherical on laying is, however, illustrated by Tsalolikhin (1983) in his book. Since eggs of these species can not be identified unless laid from an identified female, their shape for taxonomic purposes is listed in the description of the genus *Eutobrilus* as oval (Tsalolikhin 1983). Furthermore, since fixation leads to shrinkage, measurements from fixed material will always be lower than those for unfixed, freshly laid eggs.

The combination of characters described above places these worms firmly in the Tobrilidae. Minor differences between the populations in South Australia and South Africa are not considered significant and the worms are confidently referred to as *E. heptapapillatus*.

Much remains to be learnt about this cosmopolitan nematode. Its feeding habits, rates of growth and longevity in the lakes are unknown. Such information is needed if its value as an indicator of environmental pollution in these environments is to be determined.

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